Pyrolysis of Tobacco Extracts

W. S. Schlotzhauer[1], E. Barr Higman[1] and I. Schmeltz[2]

[1]Richard B. Russell Agricultural Research Center, ARS, USDA, Athens, Ga. 30604 and [2]Eastern Marketing and Nutrition Research Division, ARS, USDA, Philadelphia, Pa. 19118.

In discussing the role of pyrolysis in tobacco research, we shall attempt to review briefly the past history of pyrolysis experiments in smoking and health related studies, and to present in some greater detail data obtained from several representative pyrolyses of tobacco leaf extracts.

The last comprehensive review of the chemical composition of tobacco leaf and tobacco smoke lists more than 1200 individual components (1). Many of the smoke constituents are either absent in cured tobacco leaf, or present in quantities too minute to account for their presence in smoke. Obviously, these constituents are produced during the smoking process through various thermal alterations of precursor components in the cured tobacco leaf. These alterations might be of several varieties; the thermal rupture of a leaf component into smaller fragments is termed "pyrolysis", while subsequent recombination of these fragments to form new smoke components is termed "pyrosynthesis". In addition, leaf components which are transferred into the smokestream essentially unaltered may be considered "distillation" products. In the present discussion, we are concerned with the formation of new smoke constituents from cured tobacco leaf, and hence with both "pyrolysis" and "pyrosynthesis," terms which will be used interchangeably.

Many smoke constituents of biological interest, for example, the tumor-initiator benzo(a)pyrene and other polynuclear aromatic hydrocarbons (PAH) (2), and the cilia-movement inhibiting volatile phenols (3), were early recognized as arising in smoke via pyrolytic reactions. Thus, many researchers sought to identify the leaf precursors of these particular smoke constituents, and to determine

the pyrolytic mechanisms involved in their production. Some of the earlier studies were tacitly based on the assumption that one, or, at most, a few leaf components would serve as precursor for a specific smoke constituent, for example, benzo(a)pyrene. This approach was, of course, a gross oversimplification of complex processes; however, eventually some important precursor-product relationships were recognized. In this discussion, we should like to emphasize two of these relationships; first, the hexane-soluble components of tobacco leaf as pyrolytic sources of PAH (4, 5) and second, the brown leaf pigments (6) and carbohydrates (7) as sources of the volatile phenols. These two classes of smoke constituent are of particular relevance to the question of cigarette smoking and health.

In discussing the methodology of pyrolysis experiments, we shall begin with a brief description of the various parameters selected, and discuss the rationale for these selections. Most pyrolyses in tobacco research have been conducted in a chemically inert, preferably nitrogen, atmosphere. Critics of nitrogen-atmosphere pyrolysis contend that such experiments portray a false picture of the smoking process by eliminating various oxidative reactions; however, Newsome and Keith (8) demonstrated that the cigarette burning cone and immediate vicinity (as evidenced by the quantities of hydrogen, carbon monoxide, and methane present) are essentially in a reducing atmosphere. Combustion, naturally, does play a role in the smoking process; however, if the role of combustion were predominant, the major products of burning tobacco (excluding the specific alkaloids) would be carbon dioxide and water. Actually, the smoke produced can be considered the product of a very incomplete combustion.

Selection of experimental temperature is, as expected, critical in any pyrolytic study. Although the reported temperature range within a burning cigarette is wide, considering an ignition point of 300-400°C and maximum burning temperature in excess of 900°C, the majority of published pyrolysis experiments in tobacco chemistry have been performed at temperatures approaching the upper limits of this range. Touey and Mumpower (9), using thin precision thermocouple wires, determined peak burning zone temperatures to be in the vicinity of 860°C, and reported a sharp thermal gradient behind the cone, smoldering by convection. Another rationale for performing pyrolyses at relatively high temperatures was provided by observations that the carcinogenicity of tobacco pyrolysates apparently increased with experimental temperature. Wynder et al. (4) demonstrated that tobacco pyrolysates obtained at 880 and 800°C yielded tars of significantly higher carcinogenicity than those produced at 720 and 640°C. Pyrolysates obtained at 560°C failed to show activity. As further evidence of the utility of high temperature pyrolyses in smoking

and health research, the work of J. Lam (10) might be cited. Lam demonstrated that tobacco paraffins pyrolyzed at 850°C produced 3 to 10 times the yield of various PAH than that produced when the experiment was performed at 700°C; at 600°C no evidence for the formation of these compounds was obtained. In the specific experiments on pyrolysis of tobacco extracts described later in this paper in some detail, the pyrolysis temperature was 860°C.

We will discuss briefly the various apparatus utilized in pyrolysis experiments. One arrangement (6) consists of a horizontal vycor or quartz tube positioned in a tube furnace with accurate temperature control. Samples are inserted within the hot-zone of the tube in porcelain boats, and the resultant products flushed by a continual stream of nitrogen into a series of cold traps or other appropriate collection devices. This is a very fundamental arrangement; however, more elaborate systems have been described in the literature, for example, a vertical tube with sample introduced at the top and falling through the hot-zone at a controlled rate (11), or a movable furnace mechanically carried across the sample at a set speed (12). Although the researchers choice of apparatus design has varied, in each case, the principle is essentially identical. The resulting pyrolysates are generally less complex than cigarette smoke condensate, and are thereby fractionated into individual components under less drastic procedures than those required for condensate assay. Fractionation of tobacco pyrolysates, through various solvent partitionings and pH manipulations, yields ethereal solutions of neutrals, phenols, bases, and carboxylic acids suitable for gas-liquid, thin-layer, or column chromatography and various methods of spectral analysis including mass, infrared, and ultraviolet-absorption spectroscopy.

Leaving the general discussion of pyrolysis, we shall consider the specific application of this technique to smoking and health research and describe several representative studies. Ever since a British research group, headed by A. J. Lindsey (13), first confirmed the presence of PAH in cigarette smoke, considerable use has been made of pyrolytic methods to identify the leaf precursors of this class of smoke constituent. Table I lists a number of these investigations.

Paraffins were early suspected precursors of PAH. Lam demonstrated the production of at least 30 such compounds by pyrolysis of tobacco paraffins at 850°C. Gilbert and Lindsey, and Wynder and Hoffmann, found further evidence of the potency of paraffins as PAH precursors. G. M. Badger used pyrolysis data from individual paraffins, such as dotriacontane, to propose various free radical mechanisms in the pathway from aliphatic leaf components to smoke PAH. Not suprisingly, various researchers verified that the tobacco phytosterols -- including stigmasterol and β & γ-sitosterols, which contain an internal phenanthrene skeleton --

Table I. Precursors of Polynuclear Aromatic Hydrocarbons

Precursor	Reference(s)
Paraffins	Lam (10)
	Gilbert & Lindsey (2)
	Wynder & Hoffmann (14)
Dotriacontane	Badger et al. (15)
	Schlotzhauer & Schmeltz (16)
Phytosterols	Wynder & Hoffmann (14)
Stigmasterol	Badger et al. (15)
β-Sitosterol	Schlotzhauer & Schmeltz (16)
Phytol	Schlotzhauer & Schmeltz (16)
Isoprene	Gil-Av & Shabati (17)
	Oro et al. (18)

produce good yields of PAH on pyrolysis. Schlotzhauer and Schmeltz obtained significant amounts of PAH by pyrolysis of the C_{20} isoprenoid alcohol, phytol -- a tobacco leaf constituent. That the isoprenoids of tobacco leaf are important contributors of PAH in smoke is strengthened by the data of Gil-Av and Shabati, and Oro et al., that pyrolysis of isoprene alone produces large numbers of PAH; the latter group identified 64 aromatic hydrocarbons, including at least 19 PAH, in pyrolysates obtained from isoprene in hydrogen atmosphere. Experiments performed by the Agricultural Research Service, United States Department of Agriculture, sought to determine the relative effectiveness of various individual compounds toward pyrolytic production of PAH. The results for several of these test compounds are tabulated in Table II.

It is apparent from these data (Table II), that the structural characteristics of the precursor have a marked influence on the yield of PAH, for example, isoprenoid compounds being significantly

Table II. Yields of Aromatic Hydrocarbons, including PAH from Various Tobacco Leaf Constituents (16)

Compound Pyrolyzed	Structural Features	Relative Yields
Squalene	isoprenoid	2.72
Linolenic acid	unsat. fatty acid	2.21
β-Sitosterol	sterol; isoprenoid	1.62
Phytol	isoprenoid	1.55
Stearic acid	sat. fatty acid	1.25
Dotriacontane	C_{32} aliphatic	1.14
Hexane	C_6 aliphatic	1.00

ı simple aliphatic compounds tested.

L. Stedman (19) have conducted
ıe-soluble portion of flue-cured
s leaf fraction (approximately 6%
ı of aliphatic and cyclic paraffins,
sters, sterols and their esters,
es, and neutral "resins". As a
ntified as PAH precursors were seen
atively small portion of tobacco
ıvestigations were stimulated with
. (4) demonstrated the carcinogeni-
ane extract; however, Rayburn et al.
ry findings, reported no reduction
orbance at 385 mμ) in the smoke of
In an effort to clarify this issue,
o with hexane and pyrolyzed the
ıe extract, and the tobacco with
under identical conditions (860°C,
)pyrene was isolated from the neutral
ates by thin-layer chromatography
y absorbance (363,383 mμ) in cyclo-
tudy are presented in Table III.

indicate, the hexane extract of
disproportionately to the total
tobacco pyrolysate; expressed as
zed, one gram of hexane extract pro-
ıe, whereas the tobacco with hexane-
than 90 μg per gram pyrolyzed.

we felt a more exhaustive solvent
co, and pyrolysis of the fractions
ıdditional insights into precursor-
moking process. For reference, the
ured tobacco is presented in Table IV.

ction of Benzo(a)pyrene (5)

t	% Leaf	Yield BaP	% Total
g	100	5500 μg	100
g	5.6	3350 μg	61
g	94.4	2100 μg	38

Table IV. Chemical Composition of Flue-Cured Tobacco

Leaf Fraction	Constituents	% Dry Leaf Weight	
Ash	inorganics	13.5 (21)	9.25 (22)
Crude fibers	cellulose, lignin	11.2	7.34
Carbohydrates	poly & monosaccharides, starch, dextrin	22.5	36.35
Pectins	pectinic acids	8.0	8.48
Organic acids	Krebs cycle acids	12.2	9.96
Ether-solubles	oils, waxes, resins	7.3	6.61
Tannins	polyphenols	2.2	----
Nitrogen compounds	proteins, amino acids, nitrates, alkaloids	15.2	----

It is noted that the leaf consists of a preponderance of carbohydrates, crude fibers, pectins, etc., with smaller amounts of nitrogenous materials, lipids, and polyphenols. Sequential extraction of flue-cured tobacco with solvents of increasing polarity yielded the series of extracts presented in Table V. The composition of these extracts was monitored by thin-layer chromatography, and appropriate colorimetric analyses; constituents listed in the right-hand column of the table indicate an approximate order of extraction of the various classes of leaf component. Although some overlapping of components occurred, the initial three extracts, accounting for approximately 25% of dry leaf weight, essentially removed all the waxes, oils, sterols, and terpenes; ethanol extracted the brown pigments and nicotine salts, while the remaining material was largely carbohydrate.

The individual extracts and the residue were each pyrolyzed (860°C, N_2) and fractions containing hydrocarbons, phenols, and

Table V. Sequential Solvent Extraction of Flue-Cured Tobacco (23)

Solvent	% Dry Leaf Weight	Constituents
Skellysolve	7.2	Lipids, including
Chloroform	2.1	Waxes, oils,
Acetone	17.5	Sterols and terpenes.
Ethanol	12.0	Pigment, nicotine salts
Methanol	7.0	Krebs cycle acids
Water	10.7	Carbohydrates,
Residue	43.5	Cellulose, lignin, protein

Table VI. Aromatic Hydrocarbons in Pyrolysates Of Tobacco and Tobacco Extracts (23)

Benzene	Acenaphthylene
Toluene	Acenaphthene
Xylenes	Anthracene
Ethylbenzene	Phenanthrene
Styrene	Alkyl-Anthracenes
Indene	Alkyl-Phenanthrenes
Naphthalene	Fluoranthene
Alkyl-Naphthalenes	Pyrene
Biphenyl	Chrysene
Fluorene	Benzo(a)pyrene

nitrogen-containing compounds isolated, and compared with corresponding fractions obtained from pyrolysis of flue-cured tobacco. In all cases, major products obtained were qualitatively similar to those in the tobacco pyrolysate, but with significant quantitative variations. Products identified in the hydrocarbon fraction of the pyrolysates are listed in Table VI. Quantities of these aromatic hydrocarbons in the extract pyrolysates are presented in Table VII. The skellysolve through acetone extracts, removing approximately 25% of dry leaf weight and essentially all leaf lipids, accounted for 72% of the total aromatic hydrocarbon and 92% of the total benzo(a)pyrene content of a tobacco pyrolysate. The remaining 75% of the leaf is seen to produce relatively low levels of aromatic hydrocarbons on pyrolysis. Interestingly, the cumula-

Table VII. Contributions of Leaf Extracts to Levels of Aromatic Hydrocarbons in Tobacco Pyrolysate

Extract	% Dry Leaf Weight	% Contribution to Tobacco Pyrolysate	
		Total A.H.	B(a)P
Skellysolve	7.2	33.33	26.80
Chloroform	2.1	4.53	7.22
Acetone	17.5	35.37	59.28
Ethanol	12.0	2.56	< 1
Methanol	7.0	1.61	< 1
Water	10.7	2.59	< 1
Residue	43.5	16.55	7.22
Total	100	96.54	100.52

tive levels of aromatic hydrocarbons obtained by pyrolysis of the individual extracts and residue accounts for almost 97% of that obtained on pyrolysis of whole tobacco, indicating that, at least under these pyrolytic conditions, synergistic effects are negligible.

We shall next examine results of this experiment with regard to the pyrolytic yields of volatile phenols. Some background material regarding past studies of phenol precursors is presented in Table VIII.

In 1939, Wenusch first suggested quinic acid, a moiety of the chlorogenic acid ester, as a pyrolytic source of phenols. Zane and Wender pyrolyzed chlorogenic acid, rutin, and quercetin and identified a number of dihydroxy-benzene derivatives. Extensive investigations into the source of cigarette smoke phenols were performed by a group headed by A. W. Spears (cf. ref. 7). This group extracted flue-cured tobacco with hexane (7% yield) and 75% ethanol (47% yield); subsequent pyrolysis of these extracts (685°C, N_2) resulted in data indicating that the ethanol extract was a considerably more potent phenol precursor than either the hexane extract or whole tobacco. Assuming the ethanol extract to consist essentially of carbohydrate, Spears utilized C^{14}-labeled glucose in cigarettes to estimate that 41% of the smoke phenols are attributable to pyrolysis of leaf carbohydrate (this figure assumes that glucose is typical of leaf carbohydrate and that 55% of leaf is carbohydrate). Subsequent experiments performed at USDA (Table IX) indicated that a wide range of potential for pyrolytic production of phenol exists among various leaf constituents.

Table VIII. Pyrolytic Precursors of Smoke Phenols

Precursor	Reference(s)
Quinic acid	Wenusch (3)
Chlorogenic acid	Zane and Wender (24)
Carbohydrates	Bell et al. (7)
	Schlotzhauer et al. (6)
Brown pigments	Schlotzhauer et al. (6)
Lignin	Kato et al. (25)
	Schlotzhauer et al. (6)
Organic acids	Schmeltz et al. (26)

Table IX. Yields of Phenol by Pyrolysis of Leaf Constituents (6)

Constituent	mg. Phenol/ 100 g. Pyrolyzed	Relative Yield
Brown pigments	174	21.75
Lignin	104	13.00
Glucose	39	4.87
Polygalacturonic acid	29	3.62
Glucuronic acid	27	3.37
Cellulose	8	1.00

It is evident from the preceding data that carbohydrates are relatively poor precursors of phenol in comparison to lignin and brown pigments. The latter, which have been characterized by Wright et al. (27) and Chortyk et al. (28), among others, as iron-protein-chlorogenic acid-rutin complexes, produced approximately 4.5 times the yield of phenol pyrolytically than did glucose (Spears' typical carbohydrate) and more than 21 times the yield obtained from cellulose. These past observations add considerable insight toward interpretation of the data obtained from pyrolysis of the various tobacco extracts. Components identified in the pyrolysates of tobacco, and the tobacco extracts, included phenol, the isomeric cresols, and lesser amounts of xylenols. Quantitative analyses of the pyrolysates are presented in Table X. The ethanol extract and the tobacco residue account for more than 80% of the volatile phenol content of a tobacco pyrolysate. Interestingly, the ethanol extract, although only about one-fourth the weight of the residue, contributes nearly as high a proportion of these phenols as the latter. It is suggested that the 3 to 5% of brown pigments of leaf, concentrated in the ethanol extract, being considerably more potent precursors of phenols than the carbohydrates remaining in the leaf residue, account for this observation.

Table X. Contributions of Leaf Extracts to the Levels Of Volatile Phenols in Tobacco Pyrolysate (23)

Extract	% Dry Leaf Weight	% Contribution to Phenols in Tobacco Pyrolysate
Skellysolve	7.2	2.68
Chloroform	2.1	0.89
Acetone	17.5	8.03
Ethanol	12.0	38.39
Methanol	7.0	3.12
Water	10.7	2.68
Residue	43.5	43.75
Total	100	99.54

The final class of compounds to be examined in this study are the nitrogen-containing components. Because of the toxicity and high content of nicotine in cigarette smoke condensates, biological testing of such condensates and fractions thereof must be conducted on a nicotine-free basis. This is especially true for the basic fraction of smoke condensate, which Wynder and Wright (29) and Wynder and Hoffmann (30) found to be weakly tumorigenic and low in tumor-promoting activity. The presence of the carcinogenic N-heterocyclic hydrocarbons, the dibenz-acridines, in cigarette smoke has been observed by Van Duuren et al. (31). That N-heterocyclic hydrocarbons can arise from pyrolysis of nicotine has long been noted; Jarboe and Rosene (32) have reported the major pyrolysis products of nicotine to consist of a series of pyridine bases, preferably 3-substituted, plus quinoline and isoquinoline, nitrogen-containing analogs of naphthalene. In addition to the tobacco alkaloids, a variety of nitrogenous leaf components can give rise to N-heterocyclic compounds (33).

Of particular interest to smoking and health researchers is the suggestion that the secondary amines of tobacco leaf may give rise through thermal reactions to the potent tumor initiating N-nitrosamines. Recently, Hoffmann and Vais (34) have reported the isolation from cigarette smoke of five N-nitrosamines (as the corresponding hydrazones); however, evidence on the source and mode of formation of these compounds in smoke is currently incomplete.

Pyrolysis of tobacco and the various extracts give rise to pyridine, picolines, 3-ethyl-pyridine, 3-vinylpyridine, 3-cyano-pyridine (nicotinonitrile), quinolines, and benzoquinolines. In Table XI, nicotinonitrile has been quantitated since this product of thermal degradation of nicotine was a major component in all pyrolysates examined.

Table XI. Contributions of Leaf Extracts to Levels Of Nicotinonitrile in Tobacco Pyrolysate (23)

Extract	% Dry Leaf Weight	% Contribution to Tobacco Pyrolysate
Skellysolve	7.2	10.03
Chloroform	2.1	8.49
Acetone	17.5	1.93
Ethanol	12.0	30.12
Methanol	7.0	6.95
Water	10.7	1
Residue	43.5	3.09
Total	100	60.61

Although only 60% of the nicotinonitrile in a tobacco pyrolysate could be accounted for by examining the pyrolysates of the fractions listed in Table XI, half of this total was concentrated in the pyrolysate of the ethanol extract. Nicotine salts are generally extractable with alcohol; moreover, Dymicky et al. (35, 36) have implicated alkaloids and simple pyridine bases in the structural makeup of the brown pigments, which are also extractable with ethanol. The protein moieties of these pigments would also contribute to a concentration of basic products in the pyrolysate of the ethanol extract.

In summary, we have discussed the role of pyrolysis in smoking and health research; we have reviewed some of the findings of past investigations, and presented representative data obtained by pyrolysis of extracts from sequential extraction of flue-cured tobacco. Data indicated that the leaf lipids preferentially contribute to the levels of aromatic hydrocarbons, especially benzo-(a)pyrene, obtained in tobacco pyrolysis. Similar preference for pyrolytic production of volatile phenols is shown by the brown pigments and leaf carbohydrates. Bases, including N-heterocyclic compounds, largely arise from pyrolysis of nicotine of both the bound and unbound variety. This discussion on pyrolysis was limited in scope, and unfortunately, could not include all such contributions to tobacco smoke as have been made over the years by many investigators, but, hopefully, will provide a broader view as to the value of pyrolysis toward better understanding of the complex relationships between tobacco leaf and tobacco smoke.

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